

Bile acid metabolism and signalling in liver disease

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Summary:

Bile acids (BAs) serve as signalling molecules, efficiently regulating their own metabolism and transport, as well as key aspects of lipid and glucose homeostasis. BAs shape the gut microbial flora and conversely are metabolised by microbiota. Disruption of BA transport, metabolism and physiological signalling functions contribute to the pathogenesis and progression of a wide range of liver diseases including cholestatic disorders and MASLD (metabolic dysfunction-associated steatotic liver disease), as well as hepatocellular and cholangiocellular carcinoma. Additionally, impaired BA signalling may also affect the intestine and kidney, thereby contributing to failure of gut integrity and driving the progression and complications of portal hypertension, cholemic nephropathy and the development of extrahepatic malignancies such as colorectal cancer. In this review, we will summarise recent advances in the understanding of BA signalling, metabolism and transport, focusing on transcriptional regulation and novel BA-focused therapeutic strategies for cholestatic and metabolic liver diseases.

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Introduction

After their synthesis from cholesterol and conjugation with taurine or glycine in the liver, primary bile acids (BAs) are secreted into bile and further metabolised into secondary BAs by intestinal microbiota.¹ Since taurine- and glycine-conjugated BAs are negatively charged, they require active transport systems for effective cellular uptake and excretion through cell membranes on their passage between the liver and intestine, allowing BAs to circulate approximately 6–10 times a day within the enterohepatic circulation.² In addition to their detergent actions for lipid absorption in the intestine, BAs serve as ligands for several nuclear receptors (NRs) such as the farnesoid X receptor (FXR, also known as NR1H4), pregnane X receptor (PXR, NR1I2), constitutive androstane receptor (CAR, NR1I3) and vitamin D receptor (VDR, NR1I1).^{3,4} Via these NRs, BAs control their own synthesis, transport and metabolism, as well as lipid and glucose metabolism, amino acid catabolism⁵ and important aspects of innate and adaptive immunity.^{3,4} In addition to NRs, BAs signal through membrane receptors such as the G protein-coupled receptor Takeda G protein-coupled receptor 5 (TGR5; also known as GPBAR1 or MBAR),⁶ GPR39,⁷ sphingosine-1-phosphate receptor 2, muscarinic receptor M2/M3,⁸ epidermal growth factor receptor (EGFR), or alpha-5-beta-1 integrins.^{9,10} Importantly, BAs not only shape the gut microbial flora but are also metabolised by microbiota, producing novel BA metabolites and signalling molecules.¹¹ Disruption of mammalian and microbial BA metabolism and

signalling plays an important role in the pathogenesis of various liver diseases such as cholestatic disorders and metabolic dysfunction-associated steatotic liver disease (MASLD) and their progression to cirrhosis, as well as hepatic and extrahepatic malignancies. Notably, aspects related to post-transcriptional BA signalling pathways, e.g. via protein kinase C (PKC) and phosphatidylinositol 3-kinase (PI3K) have been reviewed in greater detail elsewhere^{10,12–14} and are not within the focus of the present review.

Bile acid metabolism and normal hepatic function

Hepatic synthesis, biliary secretion and enterohepatic circulation of bile acids

De novo BA synthesis from cholesterol is a multi-enzymatic pathway (Fig. 1A) regulated in a negative feedback fashion by BA-activated FXR in the liver and the ileum. In the liver, FXR-mediated activation of small heterodimer partner (SHP) represses *CYP7A1* expression as the key enzyme in BA synthesis.¹ Additionally, intestinal signalling via FXR-activated fibroblast growth factor (FGF) 15/19, which is mainly secreted from enterocytes (and to lesser extent from human hepatocytes), also inhibits *CYP7A1* expression.¹⁵ BA synthesis via *CYP7A1* (also known as the classic/neutral pathway) is responsible for the formation of the primary BAs cholic acid (CA) and chenodeoxycholic acid (CDCA).³ Sterol 27-hydrolase

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Keypoints

- Bile acids (BAs) serve as signalling molecules through dedicated receptors facilitating the communication between the liver, gut, intestinal microbiota, and the immune system in addition to their digestive function as detergents.
- As signalling molecules, BAs regulate their own metabolism and transport within the enterohepatic circulation, as well as key aspects of lipid and glucose homeostasis in the liver.
- BAs have immunomodulatory signalling effects on inflammatory pathways and immune cell function.
- Impaired BA transport and signalling in liver disease results in potentially toxic and pro-inflammatory BA levels which drive disease progression.
- Altered microbiota composition in liver diseases reduces BA diversity and impairs their normal signalling function along the gut-liver axis.
- Changes in BA metabolism and signalling contribute to fibrogenesis, portal hypertension, bacterial translocation and cholemic nephropathy.
- BA receptors and transporters are pharmacological targets for the restoration of BA homeostasis in hepatic and intestinal disorders.

(CYP27A1) constitutes the alternative/acidic pathway, contributing to CDCA synthesis.³ In addition to the liver, CYP27A1 is also expressed in adrenal glands, and some regions of the brain. In the brain, cholesterol is hydroxylated by sterol 24-hydroxylase (CYP46A1) to form 24-hydroxycholesterol, which can then be transported across the blood-brain barrier and taken up by the liver where it is converted to 7 α -24-dihydroxycholesterol by 24-hydroxycholesterol 7 α -hydroxylase (CYP39A1).¹⁶ However, BA synthesis and faecal excretion were not affected by deletion of *Cyp46a1*^{-/-} in mice.¹⁶

In mice, the *Cyp2c* cluster was identified to be responsible for BA rehydroxylation.¹⁷ *Cyp2c70* transforms CDCA into muricholic acid (6 β -hydroxylation), whereas the human orthologue CYP2C9 does not.¹⁸ *Cyp2a12* is responsible for 7 α -rehydroxylation in mice, thus it converts secondary BAs into primary BAs.¹⁹ As such, knockout of these enzymes has become a valuable tool to study the role of BAs in liver injury in mice with a humanised, more hydrophobic bile acid pool.^{20–22} Absence of protective muricholic acids and increased production of CDCA contributes to the increased hydrophobicity and cytotoxicity of the BA pool, leading to hepatic injury. Accordingly, treatment of *Cyp2c70*^{-/-} mice with hydrophilic UDCA reduced the hydrophobicity of the BA pool and normalised liver histology and hepatic injury markers.²²

The next step following BA synthesis is their conjugation with taurine or glycine via bile acyl-CoA synthetase and BAAT (BA coenzyme A amino acid N acetyltransferase), which together with their hydroxylation allows them to keep their amphipathic structure and is critical for lipid emulsification in the acidic environment of the gut.¹ Of note, it has been demonstrated in a humanised mouse model that non-parenchymal cells (NPCs) control glycine conjugation of BAs in hepatocytes.²³ The presence of human NPCs increased the expression of BAAT in human hepatocytes, establishing human NPCs as a major paracrine regulator of BA conjugation.²³

Conjugated BAs are taken up into hepatocytes via the basolateral sodium taurocholate cotransporting polypeptide (NTCP, also known as SLC10A1) and excreted into bile via the canalicular bile salt export pump (BSEP or ABCB11). Intestinal BA uptake and efflux occurs via the apical sodium-dependent BA transporter (ASBT or SLC10A2) and the organic solute

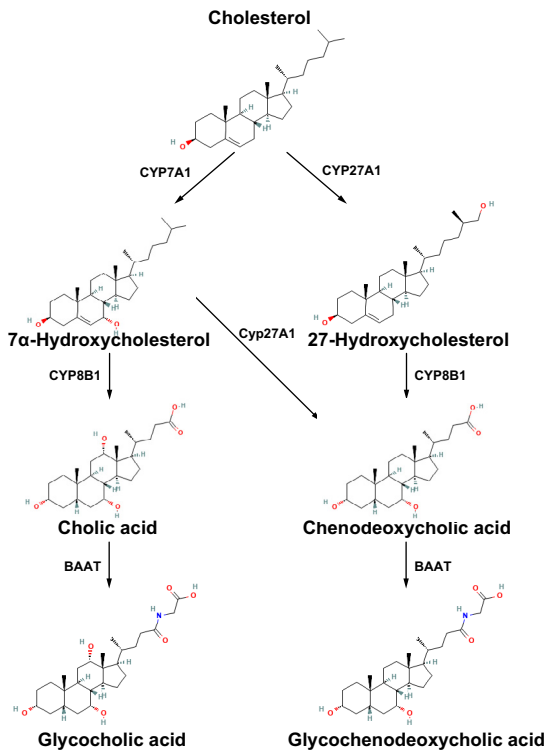
and steroid transporter (OST α/β or SLC51 α/β), respectively, thus completing the enterohepatic circulation (Fig. 2).²⁴ Unconjugated BAs are taken up by the liver via organic anion-transporting polypeptides (such as OATP1B1/SLCB1b1 and OATP1B3/SLCO1b3) (Fig. 2).²⁵ The highly efficient transport processes within the enterohepatic circulation limit daily faecal BA loss to about 3–5%. BAs escaping their ileal reabsorption reach the colon, where they are deconjugated and dehydroxylated by gut microbiota into secondary BAs (see section below).¹¹

Basolateral NTCP, the canalicular multidrug resistance-associated protein 2 (MRP2, also known as ABCC2) for conjugated organic anions such as bilirubin, and multidrug resistance associated protein-2/3 (MDR2/3, also known as ABCB4) for phospholipids, are all regulated by BAs through FXR. While NTCP is suppressed via FXR-activated SHP, the expression of the canalicular transporters (BSEP, MDR2/3 and MRP2) is directly stimulated via FXR,¹⁴ which helps to maintain BA homeostasis and restrict intracellular BA concentrations.

Lipid metabolism

BAs regulate hepatic lipid metabolism (Fig. 3). As such, FXR-induced SHP not only inhibits BA synthesis and uptake but also represses expression of *Srebp1c* (sterol regulatory element-binding protein 1c), the master regulator of *de novo* lipogenesis in mice.²⁶ In humans (but not mice) FXR activates expression of peroxisome proliferator-activated receptor- α (PPAR α /NR1C1), thereby promoting fatty acid (FA) oxidation and reducing serum triglyceride (TG) levels.²⁷ Moreover, SHP inhibits microsomal triglyceride transfer protein (MTTP, catalysing apolipoprotein B [APOB] lipidation and thereby TG export from hepatocytes) as well as APOB, resulting in reduced formation of very low-density lipoprotein (VLDL) particles. In turn, VLDL catabolism depends on lipoprotein lipase activity, which is stimulated by FXR via induction of its activator APOCII and repression of its inhibitor APOCIII. Collectively FXR stimulates VLDL catabolism, thereby increasing FA uptake in the periphery (muscle, white adipose tissue). Plasma high-density lipoprotein (HDL) cholesterol uptake is also increased by FXR-stimulated SR-B1 (scavenger receptor class B type 1)

A



B

BSH
most gram-positive, some gram-negative
i.e. *Bifidobacterium*, *Lactobacillus*, *Clostridium*,
Enterococcus, *Listeria*, *Stenotrophomonas*,
Bacteroides, and *Brucella*.

7α-dehydroxylation
some strictly anaerobic bacteria
Clostridium cluster XIV i.e. *Clostridium scindens*,
Clostridium hiranonix, *Eubacterium* species,
Faecalicatena contorta S122.

HSDH
specific bacteria
Ruminococcus gnavus, *Eggerthella lenta*

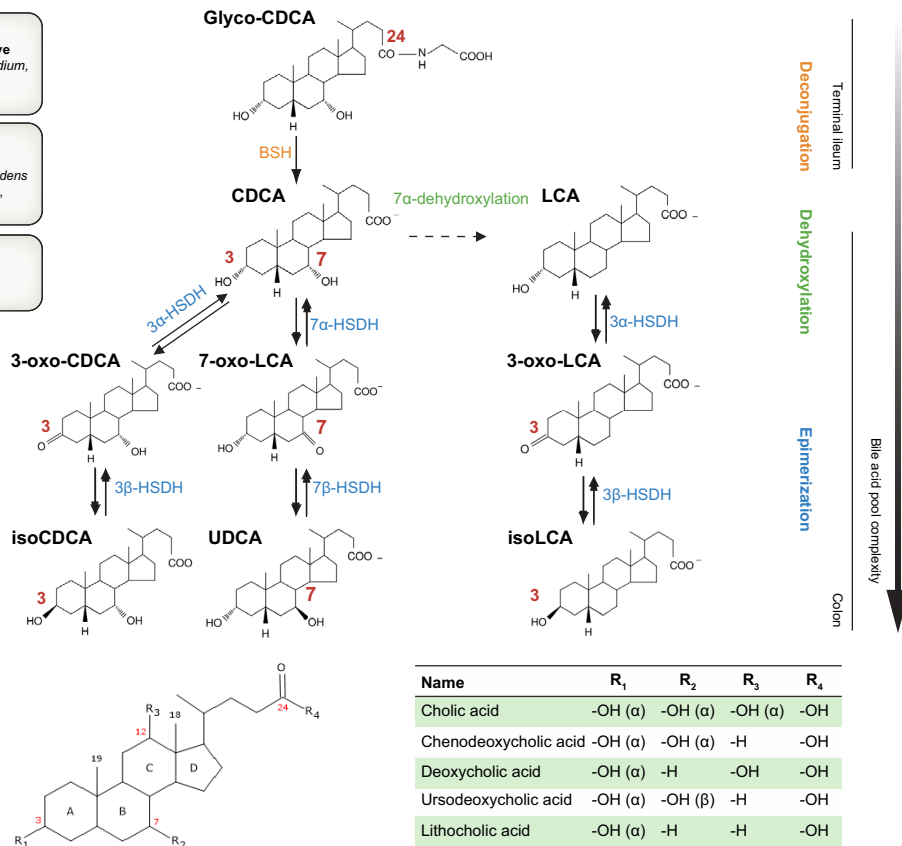


Fig. 1. Bile acid production in hepatocytes and microbial bile acid metabolism. (A) In hepatocytes, the two primary BAs — CA and CDCA — are synthesised from cholesterol via the classic (or neutral) pathway (left panel). Additionally, CDCA is also synthesised via the alternative or acidic pathway (right panel). While in the classic pathway, the enzymes CYP7A1 and CYP8B1 are involved, for the acidic pathway only CYP7B1 is relevant. After synthesis, BAs undergo conjugation with the amino acids taurine or glycine by BAAT followed by their biliary excretion. (B) Microbial BA metabolism. Dihydroxylated (CA) and trihydroxylated (CDCA) primary BAs get synthesised from cholesterol and conjugated to glycine or taurine in the liver. In the mid ileum, microbes deconjugate BAs via BSH enzymatic activity (exemplified by

expression, which (together with FXR-dependent repression of ApoA1) reduces plasma HDL levels.²⁸ In brown adipose tissue, TGR5 activates deiodinase 2, an enzyme responsible for converting thyroxine into triiodothyronine, thereby increasing energy expenditure via brown adipose tissue activation, which leads to improved insulin sensitivity and prevents diet-induced weight gain in mice.²⁹ In humans, skeletal muscle deiodinase 2 activity was detected in the presence of BAs acting as TGR5 ligands. In line, a polymorphism in the deiodinase 2 gene is associated with reduced glucose disposal rate, muscle glucose uptake, and increased insulin resistance.²⁹

Glucose metabolism

FXR agonist treatment *in vitro* (in primary hepatocytes) and *in vivo* (mouse models) stimulates the expression of phosphoenolpyruvate carboxykinase, a key enzyme in hepatic gluconeogenesis.³⁰ Other studies have shown an inhibitory function of FXR on hepatic gluconeogenesis.³¹ However, mice globally lacking FXR have reduced hepatic glycogen content³² as well as impaired glucose tolerance and insulin sensitivity.³³ Importantly, BA-FXR-FGF15/19 signalling stimulates hepatic glycogen synthesis (Fig. 3).³⁴ Collectively, these FXR-mediated effects directly or indirectly (via FGF15/19) reduce hepatic glucose output under fasting conditions and thereby improve insulin sensitivity. Furthermore, FXR as well as TGR5 expressed in β cells contribute to increased insulin secretion.³⁵ TGR5 also affects glucose homeostasis by regulating intestinal glucagon-like protein 1 (GLP-1) secretion, resulting in increased insulin secretion.³⁵ As such inhibition of ileal BA uptake (e.g. by resins, IBAT [ileal BA transporter] inhibitors) improves insulin sensitivity and diabetic control (e.g. improving HbA1c).²⁸ Accordingly, mice with TGR5 gain-of-function are more glucose tolerant, while the opposite is seen in *Tgr5* null mice. This finding was accompanied by a healthier pancreatic islet phenotype, which could be explained by increased GLP-1 secretion (induced by TGR5).⁶

Bile acids and microbiota

BAs are key molecules shaping microbial ecology in the gut.¹¹ BAs form mixed micelles that restrain bacterial density in the proximal small intestine.³⁶ In the ileum, BAs control microbial density not only via their detergent actions, but also by promoting antimicrobial peptides and inducible nitric oxide synthase via FXR-regulated pathways.³⁷ Starting in the ileum, bacteria harbouring bile salt hydrolase (*BSH*) genes carry out BA deconjugation (*i.e.*, the removal of amino acids at the C-24-position, Fig. 1B). Importantly, approximately 25% of intestinal bacteria in humans and some archaea possess BSH activity.³⁸ Bacterial diversity drastically increases in the distal gut,³⁹ where the functional groups of host-derived (primary) BAs are modified by additional bacterial enzymes resulting in a plethora of gut microbiota-derived (secondary) BAs. Deoxycholic acid (DCA) and lithocholic acid (LCA) (made from CA and CDCA, respectively) are the main microbiota-derived BAs in humans and are also the most abundant BAs in stool.⁴⁰ DCA and LCA are formed by a multistep enzymatic reaction where the

hydroxyl group at the C-7 position of an unconjugated BA is removed (7 α -dehydroxylation, Fig. 4). 7 α -dehydroxylation is carried out by a few bacteria harbouring the *bai* operon, including *C. hiranonis*, *C. scindens*, Firmicutes CAG:103 and the more recently described *Faecalicatena contorta* S122⁴¹ (Fig. 1B). In unmodified host-derived BAs, all hydroxyl groups point towards the α -side of the steroid ring, resulting in a hydrophobic α - and hydrophilic β -side. Changing hydroxyl groups from an α to a β position yields more hydrophilic iso- (C-3), urso- (C-7) or epi- (C-12) BAs.⁴² Stereoselective microbial hydroxysteroid dehydrogenases (HSDH) generating stable oxoBA intermediates carry out these reactions in both directions (Fig. 1B). Ursodeoxycholic acid (UDCA) can be isomerized back to CDCA by a 7 β -HSDH found in *Bacteroides sp.* Increased CDCA may lead to higher levels of LCA, which might explain the toxicity of UDCA treatment under certain circumstances⁴³ such as high-dose UDCA in PSC.⁴⁴ IsoBAs range from 0-20% in the stool and are epimerized back into their 3 α -hydroxy forms in the human liver.⁴⁵ 3 α - and 3 β -HSDH were initially identified in *Ruminococcus gnavus* and *Eggerthella lenta*,⁴⁶ but their poor sequence conservation hindered efforts to systemically find these enzymes in the microbiome. Compared to urso- and iso-forms, less is known about epiBAs. Nonetheless, 12-oxoLCA formed from the 12 α -HSDH oxidation of DCA can reach concentrations half of the latter.

Microbial BA metabolism is part of an arms race to harvest energy and generate molecules that are toxic to competing bacteria. Oxygen is limiting in the colon and BAs provide energetic benefits to bacteria by serving as electron acceptors. Additionally, distinct bacterial species can be more sensitive to specific BAs. For example, 3 β -isomerization of microbiota-derived BAs reduces their hydrophobicity and consequentially their toxicity towards *Bacteroides sp.*⁴⁶ Increased DCA and LCA levels inhibit the vegetative growth of *Clostridioides difficile*; however, high levels of LCA induce a morphotype switch associated with persistence in another pathogen, vancomycin-resistant *Enterococcus*, thus highlighting the complex effects of BAs on bacterial physiology.⁴⁷ These effects might become particularly relevant in disease settings such as PSC where vancomycin has been evaluated as a potential treatment in paediatric and adult patients,⁴⁸ particularly since UDCA can promote colonic LCA enrichment.⁴⁴ However, the frequency of vancomycin-resistant *Enterococcus* infections in these patients remains to be further investigated. Frequently occurring alterations in BA synthesis or excretion in liver disease influence microbiome composition, implying an intricate bidirectional interplay with BAs serving as messengers. Microbiota 'dysbiosis' describes altered microbiome composition in diseased compared to healthy individuals. Increased luminal oxygen levels are a potential pathomechanism, as reduced diversity with overgrowth of facultative anaerobes and vanishing obligate fermenters (including 7 α -dehydroxylating species) is a common feature of dysbiosis. Such reduced metabolic capacity leads to increased levels of conjugated and host-derived BAs, while microbiota-derived BAs are reduced. Dysbiosis with

Glyco-CDCA). 7 α -dehydroxylation, yields LCA (from CDCA) and DCA (from CA). HSDH can further modify primary or secondary BAs via oxidation and reduction of hydroxyl groups in a stereospecific manner, thereby generating stable oxo-BA intermediates or flipping their orientation from the α to β position, yielding isoBA (C3) or ursoBA (C7). BA, bile acids; BAAT, coenzyme A:amino acid N-acyltransferase; BSH, bile salt hydrolase; CA, cholic acid; CDCA, chenodeoxycholic acid; HSDH, hydroxysteroid dehydrogenases; LCA, lithocholic acid.

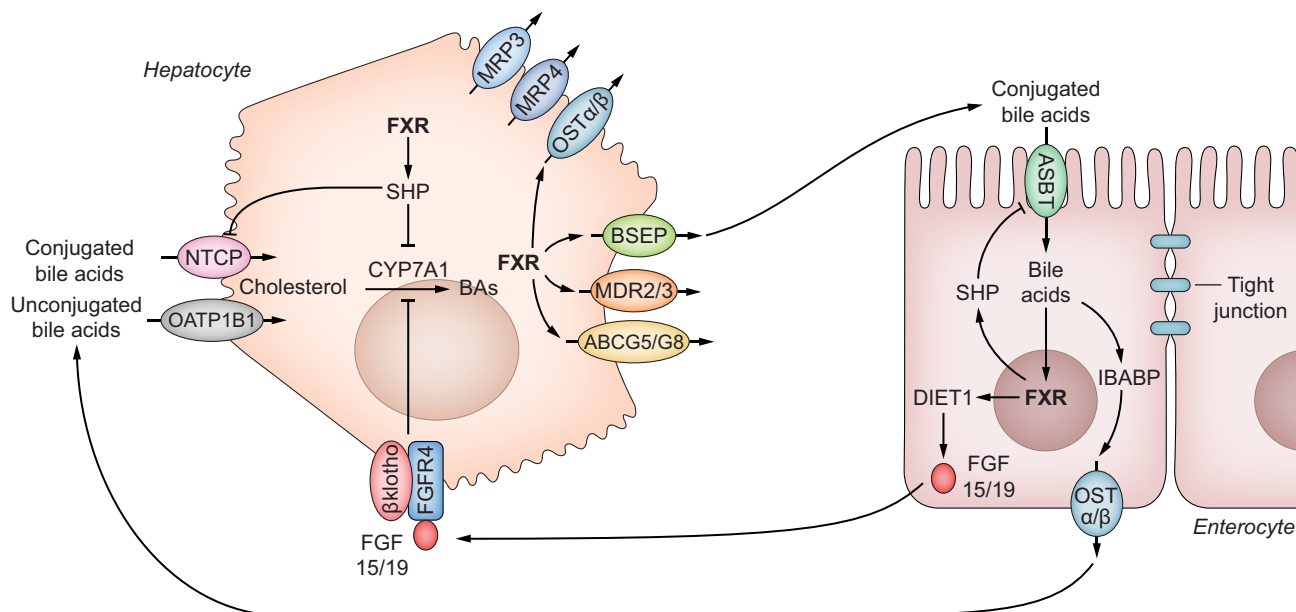


Fig. 2. Enterohepatic circulation of bile acids. In the liver, FXR inhibits CYP7A1 in a SHP-dependent manner. FXR induces canalicular BSEP, which actively secretes conjugated BAs into bile as well as MDR2/3 (phospholipid export) and ABCG5/8 (cholesterol export). MRP3, MRP4 as well as OST α/β facilitate the alternative hepatic BA export route. In the intestine, BAs are taken up via ASBT. Intestinal FXR induces the sinusoidal OST α/β heterodimer to efflux BAs into the portal blood for circulation to the liver, where they are reabsorbed via NTCP (conjugated BAs) and OATP 1B1 and 1B3 (unconjugated BAs) into hepatocytes. Intestinal FXR induces FGF15/19. DIET1 promotes FGF15/19 secretion to the portal blood, which circulates to the liver, where FGF15/19 binds to its receptor FGFR4/ β -klotho, subsequently inhibiting BA synthesis. ASBT, apical sodium-dependent BA transporter; BA, bile acid; BSEP, bile salt export pump; FGF15/19, fibroblast growth factor 15/19; FXR, farnesoid X receptor; MDR2/3, multidrug resistance-associated protein 2/3; MRP, multidrugresistance-related protein; NTCP, sodium taurocholate cotransporting polypeptide; OATP, organic anion-transporting polypeptides; OST α/β , organic solute transporter- α/β .

decreased microbiota-derived BAs has been described in various liver diseases such as in PSC,⁴⁹ MASLD,⁵⁰ and in the progression of liver disease to advanced chronic liver disease (ACLD).⁵¹ Dysbiosis-associated changes in the BA pool directly influence hepatic signalling pathways via the gut-liver axis.³

Iatrogenic modulation of the BA pool exerts broad effects on the intestinal microbiome. Suppression of BA synthesis by obeticholic acid (OCA) leads to an expansion of gram-positive strains including *Lactobacillus Bifidobacterium* and *Lactococcus* species. Conversely, supplementation with CA decreases the abundance of *Lactobacillus* and *Ruminococcus* species while fostering the growth of gram-negative pathogens including *Prevotella* sp. and *Desulfovibrio* sp., as demonstrated in mice.⁵² In healthy volunteers, supplementation with UDCA led to an expansion of *Faecalibacterium prausnitzii* which was associated with a decrease in *R. gnavus*.⁵³ Since bacteria modify BAs, it remains unclear which specific molecules mediate such effects in complex microbial communities. Recent research mainly focuses on colonic/faecal microbiota, while there is much to discover in the small intestine, where new BA species (Phe, Tyr, Leu conjugates) that have TGR5 and FXR agonistic functions, were recently found.^{54,55} A recent study revealed that BSH can also contribute to formation of bacterial BA amides.³⁴ These amidated BAs are also found in low levels in human bile, where they present a rather minor portion of total BAs.⁵⁶ Another study identified BAs amidated with polyamines whose patterns can be altered by dietary changes.⁵⁷ However, the biologic function of these new BA species remains to be determined.

Bile acids in inflammation and immunity

BA metabolism has emerged as a key determinant of immune homeostasis due to its profound effects on T cells and other cells of the innate and adaptive immune system (Fig. 4). An adequate balance between antimicrobial T helper 17 (Th17) cells and anti-inflammatory regulatory T (Treg) cells ensures intestinal barrier integrity, nutrient absorption, microbiome diversity, and gastrointestinal health. Several microbiota-derived BAs were shown to skew CD4⁺ T cell polarisation towards the Treg or Th17 cell lineage. 3-OxoLCA and isoLCA limited the generation of Th17 cells by directly binding to ROR γ t, a nuclear receptor essential for their development. Furthermore, 3OxoLCA modulated ROR γ t⁺ Treg cells through the BA receptor VDR.⁵⁸ The role of VDR in modulating ROR γ t⁺ Treg cells raises the possibility that human VDR genetic variants associated with inflammatory bowel disease (IBD) might affect disease susceptibility through improper control of the intestinal Treg cell pool.⁵⁸

Another LCA derivative, isoalloLCA, enhanced Treg cell differentiation by inducing mitochondrial reactive oxygen species.⁵⁹ Although this effect was later found to depend on NUR77/NR4A1,⁶⁰ the identity of targets directly engaged by isoalloLCA remains unknown. In mice, administration of 3-oxoLCA together with isoalloLCA reduced colonic Th17 to Treg cell ratios, but neither BA showed these effects on its own.⁵⁸ Other microbiota-derived BAs including omega muricholic acid (ω MCA) and isoDCA also boost Treg cell differentiation. isoDCA imprinted an anti-inflammatory phenotype in dendritic cells by antagonising Fxr activity, thus indirectly facilitating Foxp3 (forkhead box P3) expression in naïve CD4⁺ T

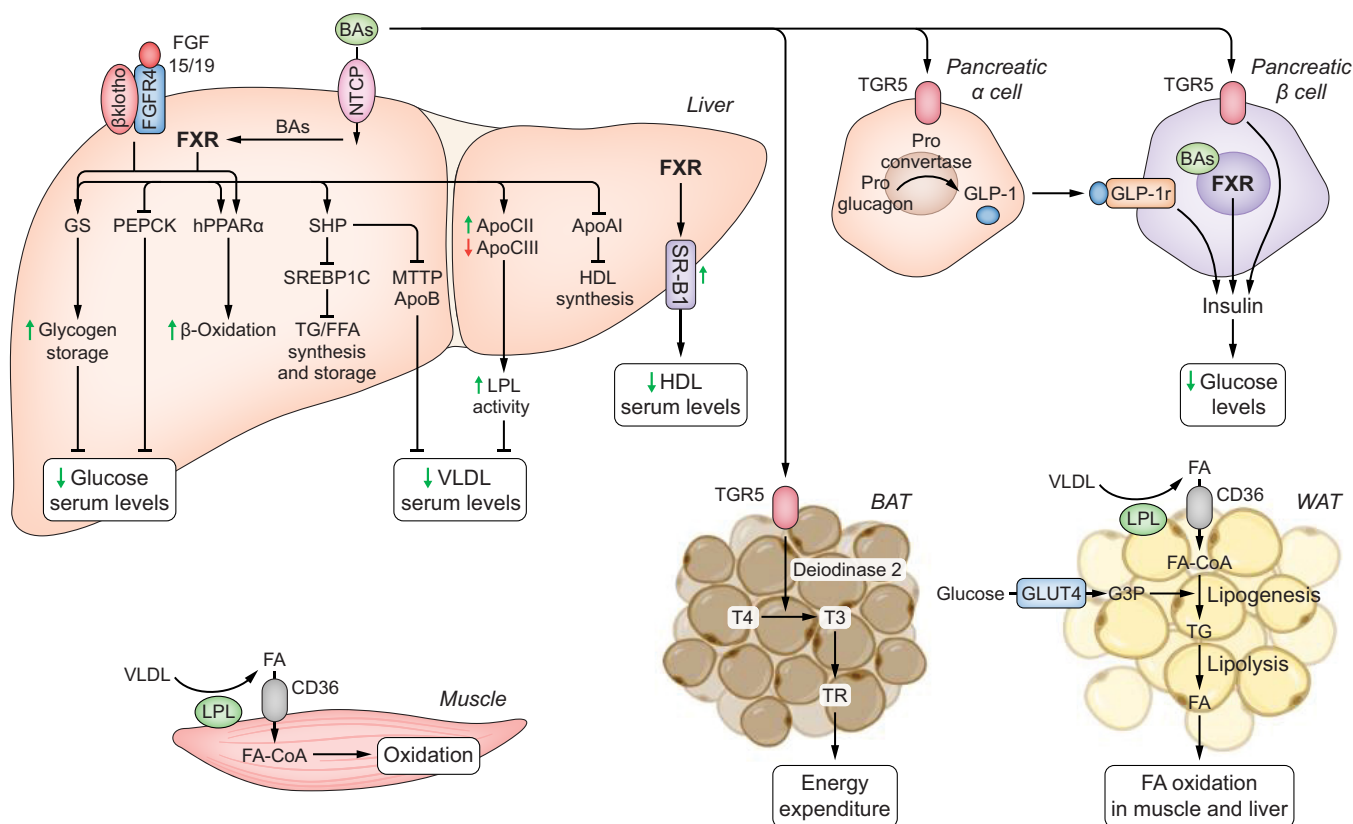


Fig. 3. Impact of bile acids on lipid and glucose metabolism. Activation of FXR by BAs results in increased glycogen synthesis and storage as well as suppression of PEPCK, a key enzyme in gluconeogenesis, thus reducing hepatic glucose output. FGF15/19 signalling also contributes to glycogen storage by promoting glycogen synthesis via activation of glycogen synthase and inhibiting gluconeogenesis. Activation of FXR lowers plasma triglyceride levels via repression of hepatic transcription factor SREBP1c expression and its lipogenic target genes. In addition, via SHP, FXR also represses *MTTP* and *APOB* gene expression and thereby the production of VLDL particles. *MTTP* catalyses ApoB lipidation using FAs, triglycerides and cholesterol to produce VLDL particles. VLDL catabolism depends on LPL activity. FXR induces ApoCII, an activator of LPL, and represses the LPL inhibitor ApoCIII. FXR stimulates LPL activity and VLDL catabolism, thus increasing FA uptake in the periphery (muscle, white adipose tissue). Furthermore, in humans but not in mice, FXR upregulates PPARα expression thereby stimulating β-oxidation and FA consumption in the liver, which counteracts fatty liver development. Plasma HDL cholesterol uptake is also increased by FXR-stimulated SR-B1 expression, further reducing plasma HDL levels (together with FXR-dependent repression of ApoA1). VLDL particles circulating in the bloodstream are hydrolyzed via LPL and the FAs are taken up via CD36 into skeletal muscle or WAT. In the muscle, FA may undergo oxidation (or re-esterification for storage as TGs). In WAT FAs and G3P are converted into TGs via lipogenesis for storage. TG breakdown (Lipolysis) releases FAs for oxidation in muscle and liver. In BAT, BA-activated TGR5 activates D2, which converts T4 into T3, subsequently increasing energy expenditure. TGR5 activation in pancreatic α-cells initiates GLP-1 production from proglucagon via proconvertase 1 induction. In pancreatic β cells, activation of FXR and TGR5 promotes insulin secretion. BAs, bile acids; BAT, brown adipose tissue; D2, deiodinase 2; FA, fatty acid; FGF15/19, fibroblast growth factor 15/19; FXR, farnesoid X receptor; G3P, glycerol-3-phosphate; GS, glycogen synthase; HDL, high-density lipoprotein; hPPARα, human PPARα; LPL, lipoprotein lipase; NTCP, sodium taurocholate cotransporting polypeptide; T3, triiodothyronine; T4, thyroxine; TG, triglyceride; TR, thyroid hormone receptor; PEPCK, phosphoenolpyruvate carboxykinase; VLDL, very low-density lipoprotein; WAT, white adipose tissue.

cells.⁶¹ Colonisation of germ-free mice with an isoDCA-producing consortium boosted colonic Treg cells compared to control bacteria lacking the relevant enzymatic activity. In addition to Treg cell differentiation, BAs also regulate their cellular fitness and function. Experimental conditional ablation of the Vdr in Treg cells reduced their frequencies in the colon, altered the expression of immunosuppressive genes and increased susceptibility to colitis. These effects were unrelated to vitamin D intake, suggesting that activation of Vdr by LCA derivatives, such as 3-oxoLCA and isoalloLCA, is required for its anti-inflammatory effects.

BA receptors are also highly expressed in innate immune cells and serve immunoregulatory functions in various myeloid cell types.⁶² Fxr activation in monocytes and macrophages isolated from the liver and intestine attenuated their pro-inflammatory

activity.³ Further, Fxr-mediated suppression of interleukin (IL)-1β and tumour necrosis factor-α (TNF-α) production by hepatic macrophages limits Th1/Th17 polarisation and the progression of sclerosing cholangitis in a mouse model.⁶³ Interestingly, Fxr also inhibits NF-κB⁶⁴ as well as Toll like receptor 9-dependent inflammation driven by gut macrophages,⁶⁵ suggesting a major role for this NR in regulating hepatic and intestinal immunity. Moreover, Car was identified to regulate Mdr1 as well as Cyp2b10 expression in T cells in the small intestine, helping them to adapt to high intestinal BA concentrations and counteract inflammation by promoting BA detoxification and producing anti-inflammatory cytokines such as IL-10.⁶⁶

TGR5 is widely expressed in human, rabbit and bovine macrophages.⁶⁷ Its activation by either host- or microbiota-derived BAs (LCA>DCA>CDCA>CA) leads to a cAMP-mediated

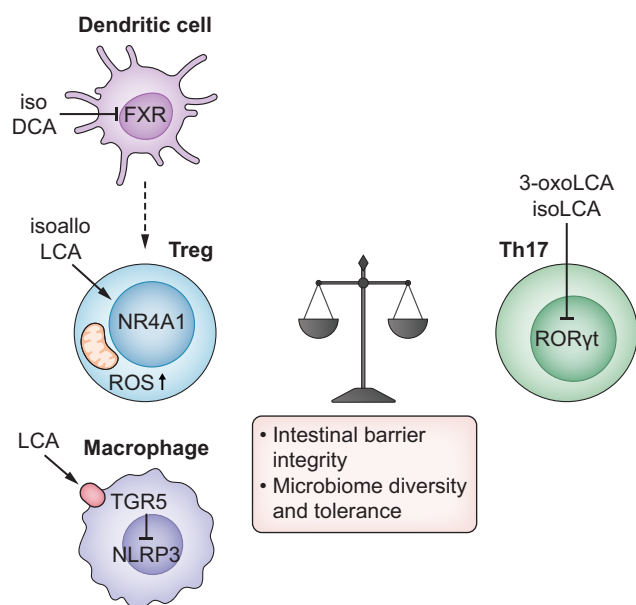


Fig. 4. Immunoregulatory effects of secondary bile acids. 3-oxoLCA and isoLCA limit Th17 differentiation by antagonising the transcription factor ROR γ t. IsoalloLCA and isoDCA promote Treg cell differentiation via direct and indirect mechanisms, respectively. IsoDCA antagonises FXR activation in dendritic cells, while isoalloLCA induces Foxp3 expression in an NR4A1-dependent manner by increasing mitochondrial ROS levels. LCA is decreased during colitis and can reverse intestinal inflammation by activating TGR5 in various immune cell types, inducing an anti-inflammatory profile in macrophages. BAs, bile acids; DCA, deoxycholic acid; Foxp3, forkhead box P3; FXR, farnesoid X receptor; LCA, lithocholic acid; ROR γ t, RAR-related orphan receptor γ t; ROS, reactive oxygen species; TGR5, Takeda G protein-coupled receptor 5.

reduction in phagocytosis, NF- κ B and NLRP3 signalling and cytokine production upon inflammatory stimulation.^{68,69} Moreover, Tgr5 activation by DCA limits the secretion of TNF α , IFN γ , IL-6, and IL-1 β in murine colonic macrophages, promoting their differentiation into an IL-10-secreting phenotype.⁷⁰

Hydrophobic BAs such as CDCA also play a putative role in initiating inflammatory responses by inducing neutrophil recruitment and cytokine release from Kupffer cells.^{71,72} DCA and CDCA upregulate the transcription factor early growth factor 1 (Egr1),⁷³ leading to pro-inflammatory cytokine secretion in hepatocytes. TCA induces IL-23 in murine hepatocytes via the activation of the Akt/Jnk signalling cascade, thereby also contributing to a pro-inflammatory Th17 cell response.⁷⁴ Conversely, polyhydroxylated BAs (especially tetrahydroxylated BAs) were shown to counteract Egr1-related cytokine secretion from hepatocytes.⁷⁵ In sum, BAs are key signalling molecules regulating host immunity and metabolism. BA transformation by bacteria relays important information on microbiome status to the host and holds great therapeutic potential.

Bile acid metabolism and signalling in pathogenesis of liver disease and therapeutic implications

Cholestatic liver disease

Cholestasis is characterised by impaired excretion of BAs, resulting in their intrahepatic and systemic accumulation.⁷⁶

Under cholestatic conditions, high BA concentrations damage the liver through their pro-apoptotic and pro-inflammatory actions.⁷⁷ In addition to hereditary defects^{76,78} acquired impairment of expression and function of canalicular efflux pumps (e.g. BSEP, MRP2) is a central aspect of cholestatic liver injury.⁷⁶ Conversely, changes in hepatobiliary transporter expression and BA metabolism represent adaptive mechanisms for attenuating cholestatic injury. This includes down-regulation of the BA uptake systems NTCP and OATP1B1, in concerted action with induction of alternative basolateral efflux systems MRP3, MRP4 and OST α/β .^{79–81} Intrahepatic BA accumulation suppresses their synthesis by inhibiting CYP7A1 transcription through FXR-induced SHP.⁸² Moreover, under cholestatic (but not normal) conditions, BA-activated FXR is able to stimulate FGF19 expression in hepatocytes.⁸³ In line, patients with advanced PSC show a nearly complete suppression of BA synthesis (e.g. by FXR/FGF-19-targeted therapies) may no longer be effective.⁸⁴ Furthermore, hydroxylation, sulfation and glucuronidation of BAs reduces their toxicity and increases water solubility which – following basolateral excretion in hepatocytes – facilitates their renal elimination.³

A complex concerted action of regulatory pathways under the control of FXR and other NRs activated via BAs explains these adaptive changes in transporter expression and metabolism.^{85,86} However, in the long-term course of chronic cholestasis these adaptive mechanisms are overwhelmed by persistent bile secretory failure with accumulation of potentially toxic cholephiles. Moreover, Fxr and other NRs are down-regulated by inflammation in rodents⁸⁷ and chronic cholestatic conditions such as PBC,⁸¹ which further limits the potential for adaptation. These endogenous adaptive mechanisms may benefit from further pharmacological stimulation.

In addition to these transcriptional changes BAs have long been known to modulate bile secretion by post-transcriptional mechanisms regulating vesicular targeting (i.e. insertion/retrieval into/from cell membrane) and direct post-translational modifications (e.g. (de)phosphorylation) of transport proteins.^{14,88} As such, seminal observations such as tauro-lithocholic acid (TLCA) induced cholestasis⁸⁹ can now be explained by impairment of vesicular exocytosis (a key step for insertion of transporters into the cell membrane) via PI3K and PKC ϵ -dependent mechanisms which can be reversed by tauro-UDCA (TUDCA) and the PI3K inhibitor wortmannin.⁹⁰ Additionally, activation of α 5 β 1-integrin signalling by TUDCA⁹ may counteract cholestasis by promoting membrane insertion of Bsep and Ntcp in hepatocytes.⁹¹

Microbial diversity is reduced in patients with cholangiopathies such as PBC and even more profoundly in PSC, as a paradigmatic disease involving an altered gut-liver axis due to IBD.⁹² The changes in microbiota (dysbiosis) in PSC are distinct from IBD without PSC (e.g. *Veillonella*, pore-forming *Klebsiella pneumoniae*) and may contribute to its pathogenesis together with changes in gut permeability.⁹³ Experimental data on the role of microbiota in biliary injury have amounted to partly contradictory results: In comparison to conventionally raised NODc3c4 mice developing spontaneous biliary inflammation in intra- and extrahepatic bile ducts, germ-free NODc3c4 mice were protected from the biliary disease.⁹⁴ Moreover, cholestasis in *Mdr2*^{-/-} mice triggers intestinal

dysbiosis, intestinal barrier dysfunction and increased bacterial translocation amplifying an NLRP3 inflammasome-mediated innate immune response in the liver ⁸⁷. In contrast, the presence of microbiota in *Mdr2*^{-/-} mice protected against the development of cholestatic liver injury and fibrosis compared to germ-free *Mdr2*^{-/-} mice in another study. ⁹⁵ The findings are still incompletely understood but could be explained by changes in microbial BA metabolism. ⁹⁶ For example, microbiota changes affect BA-mediated feedback control of BA synthesis/Cyp7a1 in *Mdr2*^{-/-} mice via FXR. ⁹⁷ Moreover, *Klebsiella pneumonia* disrupts the epithelial barrier and increases susceptibility to T helper 17 (Th17) cell-mediated hepatobiliary injury in mice challenged with 3,5-diethoxycarbonyl-1,4-dihydrocollidine or bacterial-organoid co-culture systems. ⁹⁸ Interestingly, microbe-stimulated monocytes from patients with PSC drive Th17 differentiation *in vitro* and induce chemokine-mediated recruitment of Th17 cells and monocytes by cholangiocytes. ⁹⁹ The underlying functional mechanisms are only partly understood but bacterial metabolites, including BA and bilirubin species with immunomodulatory function, may be involved. For example, gut bacteria-derived BA metabolites 3-oxoLCA and isoLCA were found to suppress Th17 cell differentiation. ⁶⁰ Interestingly, a recent study linked altered intestinal microbiota composition in people with PSC to expansion of Foxp3⁺ Treg cells in the colon, possibly explaining at least in part the overall milder IBD activity in PSC, ¹⁰⁰ although the responsible mediators (e.g. BAs) remain to be identified. Characterised by an altered bile duct microbiome. Notably, biliary dysbiosis has been linked with increased concentrations of pro-inflammatory and potentially carcinogenic BAs (e.g. TLCA) in PSC. ¹⁰¹ Collectively, these data indicate that microbiota may have both protective and disease-promoting properties which may be linked to changes in microbial BA metabolism and BA signalling.

Therapeutic implications of BA signalling: UDCA and beyond

While UDCA, is the established first-line therapy for cholestatic disorders such as PBC and intrahepatic cholestasis of pregnancy, the evidence for its long-term efficacy in PSC and a range of other cholestatic disorders is still limited. ^{102–104} The anti-cholestatic mechanisms of action of UDCA (reviewed elsewhere ¹⁰²) are mediated mostly at the post-transcriptional level through activation of PKC α , mitogen-activated protein kinases (MAPK: Erk1/2, p38^{MAPK}) and $\alpha_5\beta_1$ integrins, resulting in vesicular targeting of transporters to the cell membrane and restoration of hepatobiliary excretory function. ¹⁰² Of note, *in vivo* UDCA acted as a weak FXR antagonist in obese patients with MASLD. ¹⁰⁵ In line with its central function in BA homeostasis, FXR is a central therapeutic target in cholestatic liver diseases. In addition, FXR also modulates inflammation, intestinal integrity and microbiota, fibrogenesis and carcinogenesis. ³ OCA (first-in-class steroidal FXR agonist) was conditionally approved as a second-line therapy for patients with PBC inadequately responding to UDCA. ¹⁰³ The main side effect of OCA in both PBC and PSC is pruritus which marks a relevant reason for treatment discontinuation. ^{106,107} In addition to biochemical and some evidence for histological improvement, ¹⁰⁸ patients treated with OCA showed a significantly increased transplant-free survival compared to real-world

external controls. ¹⁰⁹ However, the negative results of the phase IV COBALT study in PBC (NCT02308111) recently led to the European Medicines Agency's recommendation to revoke the marketing authorisation for OCA.

In addition to the steroidal FXR agonist OCA, several non-steroidal FXR agonists are currently under investigation. ¹¹⁰ Some of these compounds show gut-selective or preferential action and shorter hepatic and systemic exposure, raising expectations for a better safety profile and reduced side effects, namely pruritus and dyslipidaemia. ¹¹¹ The gut-preferential FXR agonist cilofexor improved cholestatic liver enzymes and non-invasive markers of liver fibrosis without aggravation of pruritus in patients with PSC. ¹¹² In line, liver enzymes also improved in cilofexor-treated patients with PBC, ¹¹³ but pruritus was observed as a side effect. A phase III study of cilofexor in non-cirrhotic patients with PSC ¹¹⁴ has recently been discontinued because of the lack of anti-fibrotic effects, ¹¹⁵ despite encouraging preclinical effects in the *Mdr2*^{-/-} mouse model. ¹¹⁶ Interestingly, a recent experimental study demonstrated that systemic FXR activation may be necessary to antagonise macrophage-derived cytokine (IL1 β and TNF- α)-dependent licensing of effector T-lymphocytes and progression of sclerosing cholangitis in this model ⁵². Tropifexor, another highly potent non-steroidal FXR agonist improved gamma-glutamyltransferase (GGT) but not alkaline phosphatase (ALP) in patients with PBC, though pruritus was noted. ¹¹⁷ The lack of ALP reduction might be explained by potent FXR-related activation of ALP transcription. ¹¹⁸

Intestinal FXR signalling initiates FGF19 expression and its secretion into portal blood ¹⁵ (Fig. 2). M70/NGM282 also known as aldafermin, an FGF19 mimetic engineered not to have pro-carcinogenic properties, ¹¹⁹ reduced hepatic inflammation and fibrosis in the *Mdr2/Abcb4*^{-/-} mouse model of sclerosing cholangitis. ¹²⁰ Another FGF19 mimetic 'M52' counteracted development of hepatic fibrosis and hepatocellular carcinoma (HCC) formation in *Mdr2/Abcb4*^{-/-} and *Fxr*^{-/-} mice. ¹²¹ In patients with PSC, NGM282/aldafermin improved non-invasive serum markers of hepatic fibrosis without reducing cholestatic liver enzymes such as ALP. ¹²² Notably, serum BAs (an established marker of cholestasis), alanine aminotransferase (ALT) and aspartate aminotransferase decreased under aldafermin in this trial and patients were at least in part treated with UDCA, which may have hidden an effect on the primary endpoint ALP. ¹²² Aldafermin changed BA composition towards a more hydrophilic (less toxic) BA profile which correlated with changes in fibrosis markers in a pooled NASH and PSC population, ¹²³ suggesting that hydrophobic BAs may be a direct contributor to hepatic fibrosis. In patients with PBC inadequately responding to UDCA, NGM282/aldafermin mildly improved cholestatic liver enzymes. However, gastrointestinal side effects such as diarrhoea, abdominal pain and nausea (but not pruritus) were observed. ¹²⁴

Interruption of ileal BA reabsorption either by ASBT inhibitors or BA sequestrants facilitates faecal BA loss and reduces intrahepatic BA levels in cholestasis. ⁸ In the colon, spillover of BAs may activate TGR5 on enteroendocrine L-cells leading to secretion of GLP-1. ¹²⁵ GLP-1 entering the liver via portal blood, may bind to its receptor at cholangiocytes, thereby modulating their adaptive response to cholestasis, by reducing cholangiocyte apoptosis while at the same time favouring their proliferation, thus preventing ductopenia. ^{126,127}

The ASBT inhibitor SC-435 improved cholestatic liver injury and sclerosing cholangitis in *Mdr2*^{-/-} mice, which could be explained by a reduced biliary BA output despite stimulation of endogenous BA synthesis and suppression of FGF15 signalling.^{128,129} Furthermore, treatment with the ASBT inhibitor LUM001 (or lopixibat) decreased the frequency of KCs, neutrophils, and inflammatory monocytes accompanied by expansion of anti-inflammatory Ly6C⁻ monocytes.¹²⁸ In the *Cyp2c70*^{-/-} mouse (with a humanised BA composition consisting mainly of hydrophobic CDCA, resulting in hepatic injury),^{19,21} inhibition of Asbt with SC-435 improved liver injury independent of the hydrophobicity of the BA pool.²⁰ The ASBT inhibitors maralixibat and odevixibat effectively reduced serum BAs and pruritus in children with Alagille syndrome¹³⁰ and PFIC (progressive familial intrahepatic cholestasis),¹³¹ respectively, which has already led to their approval for these paediatric indications. Limerixibat (GSK2330672), another highly potent, non-absorbable ASBT inhibitor, reduced pruritus in patients with PBC.^{132,133} However, a phase II clinical trial of maralixibat improved pruritus in PSC¹³⁴ but not PBC.¹³⁵ Systemic ASBT inhibitors acting in both the ileum and kidney may be even more effective since increased urinary BA excretion through renal ASBT inhibition additionally facilitates BA loss under cholestatic conditions with an impaired enterohepatic BA circulation.¹³⁶

NTCP inhibition with myrcludex B (bulevirtide), a small peptide inhibitor used to prevent HBV entry via NTCP (see below) may also reduce intrahepatic BA levels.¹³⁷ Myrcludex B improved hepatic inflammation and fibrosis in a mouse model of sclerosing cholangitis.¹³⁸ However, targeting NTCP to attenuate BA-mediated activation of hepatic stellate cells (HSCs) is controversial.^{139,140} It has been suggested that conjugated BAs cannot be taken up by HSCs due to the lack of sufficient expression of uptake systems such as NTCP.¹⁴¹ Rather it has been demonstrated that BAs only induce proliferation of activated HSCs, via activation of the EGFR, but do not increase mRNA levels of collagen type I and TGF- β .¹⁴¹ These controversial findings could at least in part be attributed to the observation that NTCP expression in HSCs increases with the grade of hepatic fibrosis as well as with their activation status.¹³⁹ Moreover, activation of FXR (known to suppress NTCP expression) e.g. via OCA also reduced NTCP expression in HSCs.¹³⁹

While loss of *BSEP/ABCB11* causes severe cholestatic liver injury in humans, *Bsep*^{-/-} mice are protected from acquired cholestatic liver injury. This surprising observation may be mechanistically explained by metabolic preconditioning with a less toxic bile pool mainly consisting of polyhydroxylated BAs.¹⁴² Furthermore, loss of *Bsep* as well as feeding a tetrahydroxylated BAs protects *Mdr2*^{-/-} mice from the development of cholestatic liver disease⁷⁵ via repression of the pro-inflammatory regulator Egr1. Despite these promising preclinical findings, therapeutic benefits of polyhydroxylated BAs still need to be explored.

Nor-ursodeoxycholic acid (*norUDCA*, recently renamed as norucholic acid/NCA), a side chain-shortened derivative of UDCA, is resistant to side-chain conjugation with glycine and taurine.¹⁴³ In contrast to unconjugated BAs, their corresponding conjugated derivatives are fully ionised at physiological pH levels.¹⁴⁴ As such they are membrane impermeable and active BA transport systems are necessary to maintain the

enterohepatic circulation of BAs. Due to conjugation resistance, *norUDCA* — in contrast to UDCA — can be passively transported into cholangiocytes and re-secreted into the canaliculus, thereby completing a cholehepatic shunting between cholangiocytes and hepatocytes, leading to the generation of a HCO₃⁻ rich hyperchloresis. More specifically, *norUDCA* is actively secreted in its anionic form into bile where it is converted into the protonated form by accepting a hydrogen cation derived from ionisation of carbonic acid. This step also generates HCO₃⁻ and results in increased alkalinisation of the bile¹⁴³ which is a key protective mechanism (“HCO₃⁻ umbrella”) against BA toxicity to cholangiocytes,¹⁴⁵ preventing uncontrolled membrane permeation of (glycine-conjugated) BAs.¹⁰² In addition to the traditional concept of cholehepatic shunting, HCO₃⁻ secretion can be activated via activation of the calcium-activated chloride channel transmembrane member 16A (TMEM16A, also named Anoctamin-1, ANO1). Notably, conjugated UDCA is also able to activate TMEM16A, but in contrast to *norUDCA* this effect depends on active transport into cholangiocytes via ASBT.¹⁴⁶ Moreover, *norUDCA* restores *Tgr5* expression on cholangiocytes, thus potentially amplifying the *norUDCA*-related increase in biliary HCO₃⁻ secretion which is also regulated by TGR5.¹⁴⁷ In addition, *norUDCA* attenuates CD8⁺-related hepatic immunopathology¹⁴⁸ and does not act via FXR or other NRs,¹⁴⁹ making it an attractive combination partner for drugs targeting NRs within the enterohepatic circulation. In line with beneficial preclinical effects in the *Mdr2/Abcb4*^{-/-} mouse model of sclerosing cholangitis,¹⁵⁰ improved biochemical markers of cholestasis irrespective of prior exposure and response to UDCA were seen in patients with PSC subjected to *norUDCA*.¹⁵¹ A phase III trial investigating *norUDCA* treatment in patients with PSC is ongoing (NCT03872921), with results expected in 2024.¹⁵²

Finally, cholecystohepatic shunting of BAs may protect the liver against BA toxicity in cholestasis.¹⁵³ While most BAs are reabsorbed in the ileum, a fraction of primary BAs stored in the gallbladder can be directly absorbed by ASBT in the gallbladder and returned to the liver via the portal vein, completing a cholecystohepatic shunt, thereby preventing production of more hydrophobic and toxic secondary BAs in the gut and reducing their intrahepatic levels.¹⁵⁴ Interestingly, enlarged gallbladders (facilitating cholecystohepatic shunting) are frequently observed in patients with PSC and increased gallbladder volumes have been correlated with a less hydrophobic BA composition and lower degree of cholestasis. Moreover, cholecystectomy (disrupting cholecystohepatic shunting) aggravates cholestatic liver injury in the *Mdr2*^{-/-} mouse model and cholangiographic progression in patients with PSC.¹⁵¹ Theoretically, TGR5-mediated gallbladder dilatation^{155,156} could also be expected to have similar beneficial effects in PSC, although this phenomenon has raised concerns regarding metabolic disorders associated with increased gallstone risk.⁶ Importantly, TGR5 is reduced in PSC but overexpressed in cholangiocellular carcinoma (CCC).^{147,157} Moreover, TGR5 triggers proliferation and apoptosis resistance in malignantly transformed cholangiocytes, thus promoting CCC progression.¹⁵⁸ Finally, activation of TGR5 has also been linked to the pathogenesis of pruritus.¹⁵⁹ Collectively restoration of endogenous TGR5 signalling rather than its (over) stimulation by exogenous ligands may be a useful therapeutic strategy in PSC.¹⁴⁷

Steatotic liver disease

MASLD is driven by dysregulated glucose and lipid metabolism associated with insulin resistance and liver damage due to excessive hepatic FA uptake. As summarised above, BA homeostasis plays an essential role in these metabolic functions and therefore plays a critical role in the pathogenesis of MASLD and its progression to metabolic dysfunction-associated/nonalcoholic steatohepatitis (MASH/NASH).¹⁶⁰ Elevated levels of blood and faecal BAs, as well as an increased relative abundance of primary and conjugated serum BAs, were observed in patients with MASLD and linked to the development of MASH.^{50,161} Additionally, MASH patients showed increased C4 (metabolite reflecting BA synthesis) levels and CYP7A1 expression and reduced FGF19 serum levels.¹⁶¹ These findings indicate elevated BA production, dysfunctional FXR-FGF19 feedback signalling, and altered intestinal BA metabolism in MASLD/MASH. Accordingly, impaired hepatic FXR activation was found in patients with MASH.^{50,161} Increased free FA levels associated with MASLD were found to repress SHP activation, thus promoting increased BA synthesis and uptake through upregulated CYP7A1 and NTCP.¹⁶² Notably, increased BA synthesis in MASLD/MASH was also linked to insulin resistance.¹⁶³ Changes in BA profile towards 12 α -hydroxylated BAs correlated with plasma TG levels and insulin resistance in patients with diabetes, suggesting a critical impact of metabolic dysfunction on altered BA metabolism.¹⁶⁴ This concept is supported by transcriptomic analyses from liver biopsies in patients with MASH compared with healthy livers, suggesting a primary role of metabolic dysfunction in driving altered BA metabolism.¹⁶⁵

Alongside similar or even reduced FGF19 levels in patients with MASLD compared to healthy individuals¹⁶⁶ oral fat challenge in patients with MASLD indicated that insulin resistance is associated with impaired suppression of BA synthesis in response to FGF19.¹⁶⁷ Fgf15/19 was also shown to suppress hepatic lipogenesis through SHP and DNMT3A (DNA methyltransferase-3a, catalysing DNA methylation), a mechanism impaired in obese mice and patients with MASLD.¹⁶⁸ In brief, Fgf15/FGF19-activated SHP recruits DNMT3A to the FA synthase promoter, leading to DNA methylation and epigenetic gene repression.¹⁶⁸

These findings corroborate the presence of dysregulated BA gut-liver axis signalling in MASLD that also culminates in defective excretion of BAs (and other bile constituents). Computational bile flow modelling predicted higher pericentral biliary pressure and microcholestasis in MASLD/MASH, which is consistent with observed increases of cholestatic biomarkers in patients.¹⁶⁹ Concordantly, gene expression of the canalicular BA transporter *BSEP/ABCB11* was downregulated, while that of NTCP was upregulated in patients with MASLD.^{50,162} Genetic polymorphisms of *BSEP* have been correlated with higher serum TGs, cholesterol, and body mass index.¹⁷⁰ Finally, mice lacking *Bsep* have impaired mitochondrial FA oxidation and reduced white adipose tissue mass.¹⁷¹ However, when subjected to diet-induced steatohepatitis, these mice show less hepatic steatosis despite increased hepatic inflammation.^{172,173} These pathophysiological considerations have led to clinical investigation of different pharmacological interventions targeting BA signalling in MASLD.

Therapeutic implications

Administration of OCA improved insulin sensitivity and reduced markers of liver inflammation and fibrosis in patients with type 2 diabetes and MASLD.¹⁷⁴ A phase IIb trial in patients with MASH showed a reduction in the NAFLD activity score as well as improved hepatic fibrosis after OCA treatment.¹⁷⁵ The main side effects of OCA included pruritus and dyslipidaemia with elevation of low-density lipoprotein (LDL) cholesterol and a decrease in HDL cholesterol.¹⁷⁵ In the phase III REGENERATE trial that included patients with pre-cirrhotic MASH, OCA resulted in an improvement of fibrosis but not MASH,¹⁷⁶ again pruritus and dyslipidaemia were noted.¹⁷⁷ Notably, OCA was not approved for treatment of MASH by the FDA due to concerns regarding the benefit-risk profile in this indication.

Furthermore, the structural derivatives of GW4064 (the first-in-class isoxazole-type FXR agonist), tropifexor (LJN452)¹¹⁸ and cilofexor (GS9674 or Px201),¹⁷⁸ have also been tested in MASH.¹¹¹ Cilofexor lowered hepatic fat content (MRI-proton density fat fraction [PDFF]) and GGT in patients with MASH. Again, dose-dependent pruritus was noted¹⁷⁹ but no significant changes in lipid parameters were observed. Cilofexor was also investigated in combination with the acetyl-CoA carboxylase inhibitor firsocostat and selonsertib, an ASK1 inhibitor¹¹⁰ in patients with advanced fibrosis due to MASH. Neither monotherapy nor combination of firsocostat and cilofexor reached the primary endpoint (≥ 1 stage improvement in fibrosis without worsening of MASH). However, the combination of firsocostat and cilofexor achieved a ≥ 2 -point NAFLD activity score reduction and significant improvements in liver stiffness, ELF (enhanced liver fibrosis) score, and liver injury markers.¹¹⁰

A phase II trial of the most potent non-steroidal FXR agonist tropifexor¹¹⁸ reported an improvement of liver enzymes and hepatic fat content, while no histological improvement of fibrosis nor MASH was observed.¹⁸⁰ LDL levels were moderately increased while HDL levels declined, and pruritus was reported as a side effect.¹⁸⁰ Tropifexor was also investigated in combination with the CCR2/5 (C-C motif chemokine receptor 2/5) blocker cenicriviroc, without achieving significant histological or biochemical improvement.¹⁸¹

The non-steroidal carboxylic FXR agonist vonafexor reduced liver fat assessed by MRI-PDFF and improved liver enzymes and renal function in patients with MASH. Mild pruritus was reported as a treatment-related side effect.¹⁸² The non-steroidal, fexaramine-derived, gut-selective FXR agonist MET409 also lowered hepatic fat content¹⁸³ and is currently evaluated alone or in combination with the SGLT2 (sodium-glucose transport protein 2) inhibitor empagliflozin in patients with MASH and type 2 diabetes (NCT04702490). Again, dose-dependent pruritus and dyslipidaemia were observed as side effects.

Collectively, pruritus and dyslipidaemia were reported for both steroidal and non-steroidal FXR agonists tested in clinical trials independent of their chemical structure. In both patients with cholestatic disorders and MASH, IL-31 levels (a known pruritogen) correlated with the presence of pruritus.¹⁸⁴ OCA treatment increased circulating human IL-31 concentrations and hepatic IL-31 expression in a humanised murine model, supporting the causal relationship with FXR agonist treatment.¹⁸⁴ Another FXR-related effect is upregulation of LDL and lowering of HDL levels¹¹¹ which is seen as critical in patients with MASLD who are typically

at increased risk of cardiovascular disease. LDL increase is mechanistically attributed to elevated lipoprotein lipase activity (through apoCII upregulation) that enhances VLDL lipolysis, while inhibition of BA synthesis causes elevated intrahepatic cholesterol which impairs SREBP2 signalling, causing downregulation of LDL receptor (LDL-R) expression, and conversely, HDL production by downregulation of apolipoprotein A-I.^{185,186} The concomitant use of statins marks a potential approach to control lipid dysregulation induced by FXR agonists and FGF19 mimetics.^{187,188} Finally, FXR agonists may have a role within combination treatments for MASLD/MASH.¹⁸⁹ For example, the combination of clobexor with the GLP-1 agonist semaglutide resulted in additional improvements in steatosis (MRI-PDFF), biochemistry, and fibrosis biomarkers.¹⁹⁰

In addition to FXR, its downstream target FGF19 is an attractive therapeutic target in MASH. NGM282 significantly reduced hepatic fat content and non-invasive fibrosis markers.^{191,192} Side effects associated with NGM282 are mostly of gastrointestinal origin but also include LDL increases related to downregulated BA synthesis,^{192,193} which was improved by co-administration of rosuvastatin.¹⁸⁸ However, a phase IIb study in patients with MASH and F2 or F3 fibrosis, did not show histological improvement of fibrosis (primary endpoint), despite positive effects on secondary endpoints such as steatosis (MRI-PDFF and liver biopsy), liver enzymes, non-invasive fibrosis markers (Pro-C3 and ELF), and serum BAs.¹⁹²

Besides cholestatic liver diseases, *nor*UDCA exerts anti-inflammatory and anti-fibrotic effects in mouse models of MASH¹⁹⁴ making it a promising therapeutic agent in MASLD/MASH with encouraging phase IIa data showing a reduction of serum ALT within 12 weeks of treatment when compared with placebo.¹⁹⁵ Also, *nor*UDCA ameliorated experimental alcohol-related liver disease, possibly due to activation of PPAR γ 1,¹⁹⁶ indicating a certain potential of *nor*UDCA in alcohol-related steatotic liver disease and possibly those with MASLD who consume greater amounts of alcohol (MetALD).

ASBT inhibition has also been shown to improve hepatic steatosis in a high-fat diet mouse model by increasing faecal BAs and thereby resulting in a FXR-preferable BA profile and improved glucose tolerance.¹⁹⁷ However, the ASBT inhibitor volixibat did not yield histological or biochemical improvements in a phase II trial.¹⁹⁸ Several ASBT inhibitors are currently being tested for paediatric cholestasis, but their potential for MASH needs to be clarified (reviewed in¹⁹⁹). BA sequestrants are an alternative for pharmacological inhibition of ASBT. Resin bound BAs activate TGR5 and induce GLP-1 secretion.¹²⁵ While BA sequestrant treatment improved hepatic steatosis, inflammation and fibrosis in animal models,²⁰⁰ a clinical study did not show beneficial effects of colessevelam in patients with MASH.²⁰¹ Prolonged postprandial elevation of plasma BA levels due to genetic deletion of the hepatic BA transporter *Ntcp* in mice has been shown to increase energy expenditure and lower intestinal fat absorption,²⁰² thereby reducing hepatic steatosis. Furthermore, Hepalptide, an NTCP-specific inhibitor, has shown promise for mitigating hepatic steatosis, insulin resistance, as well as hepatic fibrosis in a murine steatohepatitis model.²⁰³

Sepsis

Hepatic dysfunction associated with sepsis and other severe inflammatory conditions ranges from acute changes, such as

cholestasis and hypoxic hepatitis, to adverse long-term outcomes such as secondary sclerosing cholangitis of critically ill patients.²⁰⁴ Elevated serum levels of bilirubin and BAs are well-known negative prognostic indicators in sepsis²⁰⁴ and are typically linked to infection with gram-negative bacteria.²⁰⁵ Downregulation of hepatocellular transport systems for BAs (NTCP, BSEP) and bilirubin (OATP, MRP2) or impaired bile secretion and bile flow at the level of small and large bile ducts may contribute to the development of cholestasis in sepsis.²⁰⁶ Lipopolysaccharide-induced pro-inflammatory cytokines (TNF, IL-1 β and IL-6) and nitric oxide are critical mediators of cholestasis in sepsis.²⁰⁶ The corresponding BA and bilirubin transporters *Ntcp* and *Mrp2* are transcriptionally downregulated as a result of reduced binding activity of the key regulatory transcription factors Hnf1 (hepatocyte nuclear factor 1) and Rxr (retinoid X receptor):Rar (retinoic acid receptor).^{207,208} The negative acute phase response to hepatic inflammation appears to be broadly associated with repression of several NRs including Rxr, Ppar and Lxr.⁸⁷ In addition to transcriptional changes in transporter expression, their retrieval from the canalicular membrane might be a relevant post-transcriptional mechanism to regulate hepatobiliary transport during endotoxemia or sepsis.²⁰⁶

Viral hepatitis

Infection with hepatotropic viruses (e.g., HBV, HCV, HDV) has been associated with increased serum BA levels.²⁰⁹ While increasing BA levels have been linked to disease severity in chronic hepatitis,^{210,211} response to antiviral treatment has been linked to decreased BA levels, indicating that BA levels may reflect disease severity and inflammation in this context.²¹² Nevertheless, BAs and BA transporters were also found to be involved in the pathogenesis of viral hepatitis, most prominently in HBV, wherein abnormal increased BA levels promote HBV replication by impairing the effector functions of CD8+ T and NK cells.²⁰⁹ NTCP marks the main hepatic uptake transporter for conjugated BAs and functions as an entry receptor for HBV and its satellite virus HDV.²¹³ Both viruses are internalised via interaction between the PreS1 domains of their large surface protein and NTCP.^{214,215} Interestingly, genetic variants of NTCP exhibit protective effects against HBV and HDV infection.²¹⁶ Genetic lack of *NTCP* in mice and men results in increased serum levels of conjugated BAs in the blood, reaching cholestatic levels without relevant liver injury or pruritus.²¹⁷ Naturally occurring BAs, as prototypic NTCP substrates, inhibit HBV as well as HDV infection.²¹⁸ The small peptide bulevirtide, spanning amino acids 2-48 of the PreS1 domain, acts as an NTCP inhibitor to counteract HBV and HDV infection.^{219,220} Bulevirtide has been shown to normalise serum ALT levels and suppress HDV replication after 48 weeks in patients with HDV.²²⁰ A smaller study showed that bulevirtide discontinuation upon long-term HDV RNA suppression is safe and can be successfully reinitiated in case of virologic relapse.²²¹

Advanced chronic liver disease

ACLD and its key pathological features portal hypertension (PH) and advanced fibrosis are not only driven by direct damage to the liver (e.g., alcohol or viral hepatitis) but also by gut barrier dysfunction and dysbiosis.²²² BA signalling is critically involved in regulating intestinal barrier function.²²³ Breakdown

of intestinal defence mechanisms in cirrhosis enables bacterial translocation into the portal, sinusoidal and systemic circulation that fuels inflammation, hepatic fibrogenesis, and PH through the activation of immune mechanisms responding to pathogens and their products (Fig. 5).²²⁴ Through these mechanisms, bacterial translocation promotes hepatic decompensation and acute-on-chronic liver failure. BA signalling modulates fundamental mechanisms involved in progression of ACLD and thus, offers multiple targets for therapeutic interventions.²²⁵

The steroidal FXR agonist OCA reduced intrahepatic resistance and fibrosis by ameliorating intrahepatic vascular resistance (e.g., via nitric oxide signalling), inflammation (e.g., via NF- κ B inhibition), and fibrosis (e.g., via downregulation of pro-fibrotic cytokines) in cholestatic and toxic fibrosis models.^{226,227} The non-steroidal FXR agonist cilofexor and its precursor molecule PX20606 decreased PH by improving liver

sinusoidal endothelial cell dysfunction and vascular remodeling and reduced fibrogenesis by inhibiting HSC activation in rodent models of toxic cirrhosis and MASH.^{228,229} Similar to the role of NTCP in HSCs, there has been an ongoing discussion on the direct role of FXR in HSCs and fibrogenesis.^{230,231} Recently it has been demonstrated that expression of FXR in HSCs correlates with their activation state.²³² Increased FXR signalling is capable of preserving stellate cell quiescence.²³²

BA feeding reduced bacterial overgrowth and bacterial translocation in rats with toxic cirrhosis,²³³ while bile duct ligation had the opposite effect, corresponding with reduced activation of Fxr signalling.²³⁴ Pharmacological FXR activation enhanced the secretion of antimicrobial peptides via activation of β -defensins, cathelicidin, angiopoietin-1 and CAR12, collectively maintaining intestinal homeostasis.²³⁵ Additionally, Fxr activation increases the mucous layer diameter, expression of tight junction proteins (e.g., occludin, claudin-1 and ZO-1)

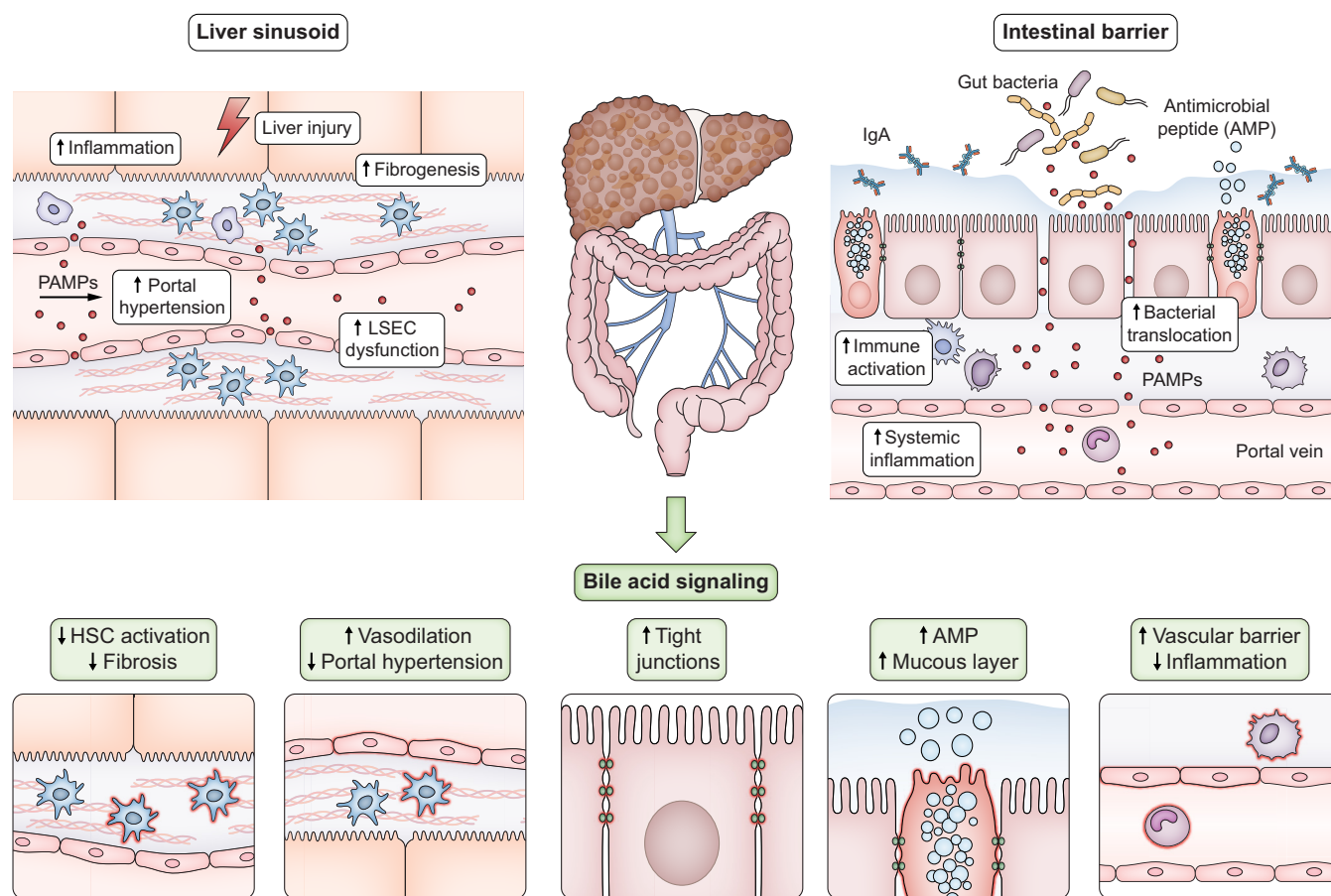


Fig. 5. Impact of bile acid signaling in advanced chronic liver disease. Liver sinusoid: Liver damage and inflammatory triggers trigger the activation of HSCs, LSECs, and immune cells such as KCs. Activated HSCs produce extracellular matrix and initiate fibrogenesis, leading to a structural remodelling of the liver tissue, while dysfunctional LSECs promote a vasoconstrictive phenotype and initiate sinusoidal capillarization, leading to an increased vascular tone in the liver sinusoid. These changes cause an impaired microcirculation in the liver sinusoid that further aggravates hepatic dysfunction and portal hypertension. Intestinal barrier: Impaired integrity of the gut barrier is based on reduced mucus thickness and secretion of antimicrobial peptides, downregulation of tight junctions, and increased permeability of the gut-vascular barrier, and enables the occurrence of bacterial translocation. Translocated bacteria and bacterial products are recognized by immune cells in the gut, systemic circulation, and the liver. The resulting inflammatory response induces systemic inflammation and promotes fibrogenesis, portal hypertension, and disease progression in ACLD. BA signalling modulates key pathomechanisms in ACLD: In the liver, FXR signalling inhibits the activation of HSCs (leading to reduced fibrogenesis) and LSECs (leading to reduced vasoconstriction) that may ameliorate fibrosis and portal hypertension. In the intestines, FXR activation enhances key elements forming the intestinal barrier, and thus, reduces bacterial translocation and its detrimental effects on systemic inflammation, fibrogenesis, and portal hypertension. ACLD, advanced chronic liver disease; AMP, antimicrobial peptide; BA, bile acid; FXR, farnesoid X receptor; HSCs, hepatic stellate cells; LSECs, liver sinusoidal endothelial cells; KCs, Kupffer cells; PAMPs, pathogen-associated molecular patterns.

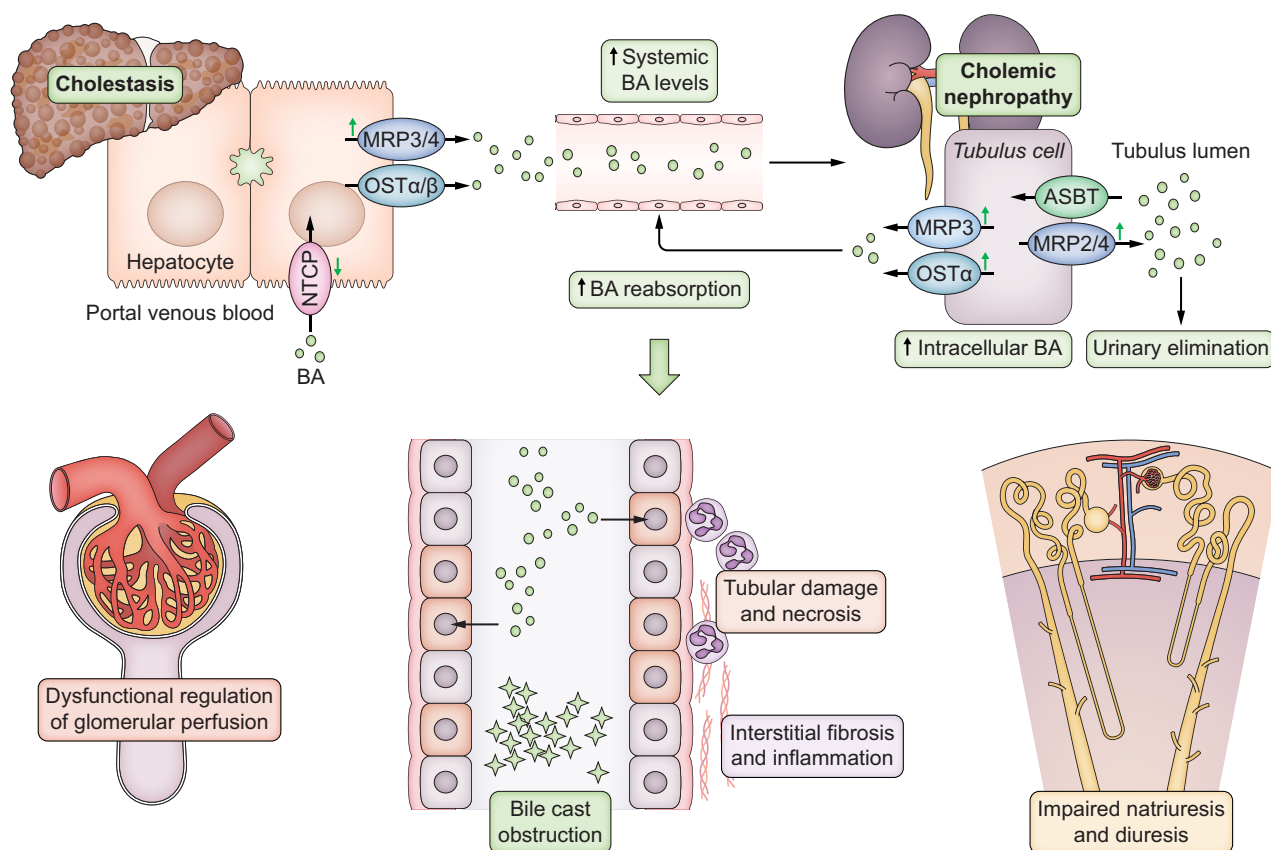


Fig. 6. Role of impaired bile acid homeostasis in cholemic nephropathy. Progressive cholestasis leads to an increased elimination of BA and bilirubin through the kidneys. The formation of bile casts at high concentrations causes dysfunctional regulation of glomerular perfusion, as well as damage, necrosis, and obstruction of renal tubuli. These conditions promote impaired tubular architecture through interstitial fibrosis and inflammation and interfere with natriuresis and diuresis both through changes in structural and physiological homeostasis. The resulting ‘cholemic nephropathy’ is considered to impact on renal function in ACLD and represents an important determinant for the response to currently available treatments in the context of hepatorenal syndrome. ACLD, advanced chronic liver disease; BA, bile acid.

maintaining epithelial barrier integrity, stabilises the gut-vascular barrier, attenuates bacterial translocation, and importantly, improves liver fibrosis and PH.^{228,236,237} Concomitantly, patients with ACLD showed impaired FXR signalling in the liver and the ileum, which corresponded with down-regulated intestinal barrier proteins.²³⁸ Nevertheless, the clinical effects of BA signalling modulation on liver fibrosis and PH in patients with ACLD remain largely unknown, because clinical trials on FXR- or FGF19-directed therapies usually excluded patients with ACLD or lacked specific readouts on PH.^{110,225} Notably, an increased risk of hepatic decompensation in patients with PBC stage IV (cirrhosis) treated with OCA was reported in a retrospective study,²³⁹ which indicates a suboptimal safety profile of steroidal FXR agonists in this setting. Likewise, the safety profile of non-steroidal FXR agonists remains understudied in ACLD and should be closely monitored during future applications.

Cholemic nephropathy, HRS-AKI

Renal dysfunction in patients with ACLD indicates worse prognosis. The differentiation between hepatorenal syndrome

(HRS) and other types of acute kidney injury (AKI) is challenging but important since underlying pathomechanisms and their clinical course are fundamentally different.²⁴⁰ A subset of renal dysfunction cases in patients with ACLD – particularly in those with jaundice – is complicated by cholemic nephropathy (CN). The pathophysiology of CN involves BA toxicity in tubular cells, obstruction of renal tubuli by bile casts, and adverse effects on glomerular perfusion and filtration (Fig. 6).^{241,242} Notably, the increased renal BA exposure in cholestasis results from adaptive hepatocellular mechanisms facilitating BA clearance through alternative basolateral escape routes.^{79–81} Response to HRS-AKI treatment is impaired in patients with high bilirubin levels which may causally relate to CN.²⁴³ Up to two-thirds of patients with acute-on-chronic liver failure, with progressive renal impairment, had histological features of CN in post-mortem kidney biopsies.²⁴⁴ Experimental studies in bile duct-ligated rodents demonstrated BA toxicity in the proximal tubular epithelium through accumulating BAs taken up by renal Asbt.^{241,242} Notably, experimental administration of NorUDCA (presumably by increasing the hydrophilicity of the BA pool) and systemic Asbt inhibition (blocking tubular uptake of urinary

excreted BAs) prevented CN in mouse models.^{241,242} Ultimately, human studies are limited by small cohort sizes and heterogeneous patient populations, warranting further research on pathomechanisms of CN in humans.

Hepatobiliary and extrahepatic malignancies in liver disease

Hydrophobic BAs promote oxidative stress and DNA damage/genomic instability, which are both predisposing factors for cancer.²⁴⁵ The high carcinogenic potential of BAs may be best exemplified by the high risk of early HCC development in patients with PFIC type 2.²⁴⁶ The major secondary BAs (DCA and LCA) have been linked to colorectal cancer (CRC)²⁴⁵ and HCC.²⁴⁷ DCA promotes DNA damage and cellular senescence in HSCs, leading to secretion of inflammatory and tumour-promoting factors, and thus creating a microenvironment that facilitates HCC development.²⁴⁸ In mice with high-fat diet- and streptozotocin-induced liver injury, enrichment of Gram-positive bacteria populations and subsequently enhanced production of DCA by 7 α -hydroxylase makes them more susceptible to HCC development. Moreover, BA depletion by cholestyramine prevented HCC development.²⁴⁸ These findings suggest that the composition of microbiota and their impact on BA metabolism may critically influence HCC risk.

Fxr controls physiological proliferation of hepatocytes in mice.²⁴⁹ Additionally, Fxr exerts anti-inflammatory effects via suppression of NF- κ B, thereby counteracting inflammation-driven carcinogenesis.⁶⁴ Mice lacking *Fxr* develop liver cancer at the age of 12–15 months.²⁵⁰ Administration of the FXR/TGR5 dual agonist (INT767) in *Mdr2*^{-/-} mice protects from liver tumour formation due to induction of intestinal Fgf15 secretion and subsequent inhibition of *Cyp7a1* expression in the liver,²⁵¹ thereby inhibiting endogenous BA synthesis and toxicity. As indicated above, the non-tumorigenic FGF19 analogue also protects against cancer.¹²¹ However, mice overexpressing the human orthologue FGF19 develop HCC²⁵² emphasising the carcinogenic potential of supraphysiological FGF19 levels. Concordantly, FGF19 was amplified in patients with HCC. Notably, inhibition of Fgfr4 attenuated HCC development in mice,²⁵³ and pilot studies showed that the Fgfr4 inhibitor FGF401 was safe in patients with HCC.²⁵⁴ Consequently, awareness regarding the long-term use of FXR agonists and their induction of supraphysiological FGF19 levels and the risk of cancer development is warranted.²⁵⁵ Since the steroidal FXR agonist OCA undergoes enterohepatic circulation,²⁵⁶ this aspect was also considered in the design of novel drugs aimed at achieving a more pulsatile (instead of a constant) FGF19 stimulation.

Cholestatic disorders such as PSC are associated with an increased risk of developing CCC,²⁵⁷ a process potentially favoured by accumulation of intrahepatic BAs. *In vitro*, BAs activate cholangiocyte proliferation via EGFR.²⁵⁸ The BA receptors TGR5 and FXR may have opposite functions in the progression of CCC. While FXR is downregulated in CCC tissue (correlating with the degree of tumour cell differentiation), TGR5 expression is upregulated in CCC tissue.²⁵⁹ *In vitro*, the FXR agonist OCA inhibits proliferation and migration of CCC while the TGR5 agonist INT-777 stimulated the proliferation, migration and metabolism of CCC cells.²⁵⁹

TGR5 activation stimulates adenylate-cyclase and increases intracellular cAMP production, subsequently activating protein kinase A and cholangiocyte proliferation via Egfr/Erk¹⁵⁸ and having anti-apoptotic effects in normal cholangiocytes¹⁵⁸ – observations arguing against the use of TGR5 agonists in cholangiopathies, such as PSC, associated with increased CCC risk. Conjugates of cisplatin with glycocholate or with UDCA have shown beneficial effects in experimental HCC models owing to their hepatotropism.²⁶⁰ A pilot study demonstrated preserved expression of ASBT in human CCC and colon cancer, which facilitated the uptake of these compounds.²⁶¹

Population-based studies have shown that people consuming a Western diet display elevated levels of faecal secondary BAs, similar to patients with CRC.²⁶² This may be of key relevance in patients with MASLD who have an increased CRC risk.²⁶³ Increased levels of secondary BAs have detrimental effects on the colonic epithelium through mechanisms involving DNA oxidative damage, inflammation, NF- κ B activation and enhanced cell proliferation.³ For example, high levels of DCA induced cell proliferation by activating epidermal EGFR and post-EGFR/ERK signalling.²⁶⁴ The chemopreventive effects of UDCA against CCC and CRC in PSC are controversial.¹⁰⁴ Small studies showed that patients with PSC receiving low and standard doses of UDCA may be at reduced risk of colonic dysplasia or CRC, while high-dose UDCA may even aggravate the risk.¹⁰⁴

Intestinal FXR expression is inversely related to the progression of CRC and the degree of malignancy.^{265,266} Constitutive activation of FXR in colon cancer cells results in suppression of colonic epithelium proliferation and induces a network of pro-apoptotic genes,²⁶⁷ indicating that loss of FXR may contribute to CRC susceptibility.

As such, targeting BA signalling could be a potential therapeutic strategy to prevent CRC formation. Application of TCA (FXR agonist) in a CRC animal model reduced tumour development due to Shp-dependent downregulation of Cyclin D1.²⁶⁸ *In vitro*, human CRC cell line (H508, SNU-C4 and HT-29) proliferation was attenuated by GW4064 via downregulation of EGFR phosphorylation and ERK activation.²⁶⁹ Furthermore, intestinal FXR activation slowed down tumour progression and prolonged survival.²⁷⁰ Accordingly, FXR expression is reduced in colorectal adenocarcinomas²⁷¹ and FXR expression in tumour tissue was inversely correlated with clinical outcomes, indicating a critical function of FXR in counteracting CRC development. In addition to more classically considered tumours in the liver and intestine, BAs and their altered signalling may also be involved in oesophageal, gastric, hepatocellular, pancreatic, breast, prostate, and ovarian cancer (reviewed in²⁷²).

Conclusion

BAs play a central role in hepatic metabolism as well as inflammation, fibrosis, and carcinogenesis. Better understanding their function in the pathophysiology of cholestatic and metabolic liver diseases is of key relevance and should allow for the therapeutic targeting of BA-related pathways. BA-targeted approaches may provide ample therapeutic opportunities for a range of liver diseases, as well as gastrointestinal disorders, along the gut-liver axis.

Affiliations

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Abbreviations

ACLD, advanced chronic liver disease; AKI, acute kidney injury; ALP, alkaline phosphatase; APOB/APOCII/APOCIII, apolipoprotein B/C-II/C-III; ASBT, apical sodium-dependent bile acid transporter; BAs, bile acids; BSEP, bile salt export pump; BSH, bile salt hydrolase; CA, cholic acid; CAR, constitutive androstane receptor; CDCA, chenodeoxycholic acid; CCC, cholangiocellular carcinoma; CN, cholemic nephropathy; CRC, colorectal cancer; CYP, cytochrome P450; DCA, deoxycholic acid; EGR1, early growth factor 1; EGFR, epidermal growth factor receptor; ELF, enhanced liver fibrosis; FA, fatty acid; FGF, fibroblast growth factor; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide-1; GGT, gamma-glutamyltransferase; HCC, hepatocellular carcinoma; HDL, high-density lipoprotein; HRS, hepatorenal syndrome; HSDH, hydroxysteroid dehydrogenases; IL-1 β /31, interleukin-1 β -31; LCA, lithocholic acid; LDL, low-density lipoprotein; MASLD, metabolic dysfunction-associated steatotic liver disease; MASH, metabolic dysfunction-associated steatohepatitis; MDR2/3, multidrug resistance associated protein-2/3; MRI-PDFF, MRI-proton density fat fraction; MRP2, multidrug resistance associated protein 2; MTTP, microsomal triglyceride transfer protein; NRS, nuclear receptors; Ntcp, sodium taurocholate cotransporting polypeptide; NUR77/NR4A1, nuclear receptor subfamily 4 group A member 1; OATP, organic anion-transporting polypeptide; OST α/β , organic solute and steroid transporter alpha/beta; PBC, primary biliary cholangitis; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PPAR α /PPAR γ 1, peroxisome proliferator-activated receptor α/γ 1; PXR, pregnane X receptor; ROS, reactive oxygen species; SHP, small heterodimer partner; SLC, solute carrier family; TCA, taurocholic acid; TG, triglyceride; TGR5, Takeda G protein-coupled receptor 5; TNF- α , tumour necrosis factor- α ; UDCA, ursodeoxycholic acid; VDR, vitamin D receptor; VLDL, very low-density lipoprotein.

Note: When referring to both mouse and human data within one statement, letters/abbreviations are displayed in the human gene/protein version.

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Conflict of interest:

CDF received travel grants from Gilead, Roche, Falk, Merck, Vifor, AbbVie, and Boehringer-Ingelheim. BeSi has received travel support from AbbVie, Gilead, and Falk. TR received grant support from AbbVie, Boehringer-Ingelheim, Gilead, MSD, Philips Healthcare, Gore; speaking honoraria from AbbVie, Gilead, Gore, Intercept, Roche, MSD; consulting/advisory board fee from AbbVie, Bayer, Boehringer-Ingelheim, Gilead, Intercept, MSD, Siemens; and travel support from AbbVie, Boehringer-Ingelheim, Gilead and Roche. MT received grant support from Albireo, Alnylam, Cymabay, Falk, Genentech, Gilead, Intercept, MSD, Takeda and UltraGenyx, honoraria for consulting from AbbVie, Albireo, Agomab, Boehringer Ingelheim, BiomX, Chemomab, Dexoligo Therapeutics, Falk, Genfit, Gilead, GSK, Hightide, Intercept, Ipsen, Janssen, MSD, Novartis, Phenex, Pliant, Regulus, Siemens and Shire, speaker fees from Albireo, Boehringer Ingelheim, Bristol-Myers Squibb, Falk, Gilead, Ipsen, Intercept, MSD and Madrigal, as well as travel support from AbbVie, Falk, Gilead, Janssen and Intercept. He is also co-inventor of patents on the medical use of 24-norursodeoxycholic acid filed by the Medical University of Graz. MB and CC report no conflicts of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

CDF and BS conceptualized and wrote the article; MB contributed to writing the article, CC and TR contributed with critical revisions; MT conceptualized the article, and reviewed and edited the manuscript before submission. All authors approved the final version of the manuscript for submission.

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Supplementary data

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